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Bait dilution, spinosad concentration, and efficacy of GF-120 based fruit fly sprays

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Abstract

We tested persistence and effects of dilution of a hydrolyzed protein edible insecticide bait for control of tropical fruit flies (Tephritidae). The bait, commercially marketed as GF-120, is a mixture of the insecticide spinosad, a microbially hydrolyzed protein, sugars, adjuvants and a series of conditioners. Bait is formulated to have both an attractant and feeding stimulant function. In experiments to determine the maximum persistence of the commercial formulation we found that if bait is protected from rain but exposed to other environmental factors (heat, sunlight, humidity) the bait remained effective for about 14 days in field cages. In a laboratory cage test experiment to determine the effects of spinosad concentration in the commercial bait after exposure to field conditions, concentrations of 8 mg AI l^{-1} bait did not differ from the control (no spinosad). Concentrations of 80 mg l^{-1} had significantly lower knockdown (kill flies within 2 h) than 800 mg l^{-1}, but the two concentrations did not significantly differ in numbers of survivors over a 4 day treatment period time. Field cage tests showed significant differences among spinosad concentrations and bait ages for knockdown of flies but 80 and 800 mg spinosad l^{-1} were similar for rates of survival over 4 day tests. A final test was performed to measure the effects of bait + insecticide dilution on function of the bait after 14 days aging in the field cage. Results showed that a four-fold dilution of the complete bait did not significantly reduce attraction or knockdown. The undiluted bait was superior to eight-fold dilution but did not differ from four-fold dilution.
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Keywords: Mexican fruit fly; Spinosad; GF-120; Insecticide; Attractant; Dilution; Persistence

1. Introduction

GF-120 bait is a combination attractant, feeding stimulant, and spinosad insecticide for control of fruit fly (Tephritidae) populations. The bait was formulated to attract multiple fruit fly species and to use the minimum concentration of an environmentally compatible toxicant for ultra-low volume (2–4 l/ha) application. Spinosad was discovered and developed as an insect control product over a 17-year period from 1984 to 2001 by which time it had been registered in 50 countries on over 250 crops (Dow AgroSciences, 2001).

The bait is referred to as Solbait and is based on Solulys, a spray-dried enzymatically hydrolyzed protein that is produced from the industrial processing of corn for recovery of sugars and oil. Other additives including feeding stimulants, adjuvants, auxiliary attractants and conditioners are described in Moreno and Mangan (2002). GF-120 includes further proprietary refinements that improve the overall effectiveness.

A historic review of the development and basic chemical characteristics of spinosad is given in Thompson et al. (2000). Spinosad is a combination of spinosyn compounds that are purified from the soil actinomycete, *Saccharopolyspora spinosa* Mertz (Thompson et al.,

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2000). The major route for Spinosad degradation is photolysis. The half-life for soil photolysis is 9–10 days and anaerobic soil metabolism with absence of light is 9–17 days. Leaf surface photolysis half-life is listed as 1.6–16 days. Tests of spinosad "Success 480" formulation on apple tree leaves and exposed to light showed dissipation of spinosyn A to be 90% and spinosyn D to be 100% in 7 days compared to dissipation of 22% and 18% when applied to leaves and protected from light (Pest Management Regulatory Agency Health Canada, 2001).

The spinosad insecticide has been compared in field trials to toxic baits conventionally used against fruit flies. Burns et al. (2001) showed that spinosad mixed at 80 ppm (= 0.008% AI) in Solbait was equivalent to malathion (20% AI) in either Solbait or Nulure (the conventional bait) against Mediterranean or Caribbean fruit flies. Moreno and Mangan (2002) also showed that the 80 ppm concentration of spinosad in Solbait (1.5% survival) was equivalent to 20% malathion mixed in Nulure (1.8% survival) in comparison with untreated control plots. The GF-120 formula is registered as organic by US Department of Agriculture and international organic registries (Organic Materials Review Institute, 2002). GF-120 has been shown to have no effect on honey bees in field applications (Rendon et al., 2000). Experimental evidence has also shown that GF-120 has minimal or no effect on non-target beneficial insects (Burns et al., 2001; Vargas et al., 2001; Michaud, 2003) while the malathion–Nulure bait has been shown to have negative impacts on beneficial and pollinating insects in treated zones (Harris et al., 1980; Troetschler, 1983; Hoelmer and Dahlsten, 1993). Additional advantages of the GF-120 compared to malathion baits are that no protective cover-all clothing is required for applicators and post-spray re-entry periods are only 4 h (Specimen Label GF120 Naturalyte, 2003) compared to a minimum of 12 h for malathion on most state or national labels. Social pressures and reports of perceived or actual human health problems associated with malathion sprays (Anonymous, 1999) have led to public pressure for fly management and eradication programs to employ pesticide baits with lower mammalian toxicity and reduced active ingredient concentration in bait preparations.

Experiments with spinosad have been reported from a series of publications from research performed concurrently with the investigations reported here. Tests in Hawaii (Vargas et al., 2001, 2003; Barry et al., 2003; Stark et al., 2004; Revis et al., 2004; Prokopy et al., 2004) focused mainly on the Mediterranean fruit fly (*Ceratitis capitata*) and *Bactrocera* spp. The spinosad has also been tested for control of temperate fruit fly species of *Rhagoletis* (Reissig, 2003; Van Steenwyk and Coates, 2002; Flora et al., 1999).

Our key interest focused on bait function with respect to dilution of the bait and bait effectiveness with age (persistence). The experiments measured the maintenance of toxicity of the spray, attraction of flies to the bait, and performance of the bait for killing flies. Four separate experiments were performed. (1) Persistence of GF-120 bait applied at recommended concentrations. (2) Effect of diluting the spinosad concentration in GF-120 toxicity. (3) Effect of dilution of spinosad on GF-120 persistence. (4) Effect of dilution of total bait on persistence.

2. Materials and methods

Four experiments were carried out from 1999 to 2002, with three tests (1, 3, 4) in field cages within a grapefruit orchard. Test 2 was carried out in laboratory cages in the Crop Quality and Fruit Insect Research Unit at Weslaco. The large field cage tests were all on the USDA-ARS Weslaco Center, Hidalgo County, Texas property. Weather during these tests ranged from cool winter temperatures ranging below 15 °C at night up to 25 °C during the day, and summer temperatures of 23 °C at night up to 40 °C during the day. We attempted to perform most experiments within the range of 18–32 °C as temperatures outside these extremes reduced fly activity.

Outdoor tests were performed in two field cages, each containing four rooms, each room with one mature, and four potted nursery size, Rio Red grapefruit trees. Cages were permanent structures with wood frames anchored in concrete and with double coarse and fine steel screens on the outside walls and fabric screens separating the trees. Each field cage was 3.73 m tall, two rooms on the south side measured 6.51 m × 4.76 m. The two cages on the north side measured 4.81 m × 4.81 m. Water for the flies was provided by dental wicks extending from plastic containers in the trees. Tests were carried out by applying bait to the nursery trees outside the cages then moving them into the cages for the tests. These trees had been maintained in plastic 5-gallon pots for 3 years and were pruned to be ≈1.2 m tall and have about 50–70 leaves. Treatments to trees were made by applying 30 ml of bait per tree with a calibrated CO_2 pressurized spray gun so that spray could be directed directly onto the leaves. Spraying with the nozzle 10–20 cm from the leaf surface allowed small (0.5–1 mm diameter) drops to cover leaves at 10–20 drops per leaf. We aged the sprayed trees in an open greenhouse that was provided with circulating air at ambient temperature and humidity, then trees were moved to the field cages for testing. Sprays applied to trees were all mixed on our laboratory either by mixing the commercial GF-120 bait or in tests using different spinosad concentrations; we mixed the indgredients and added the appropriate amounts of

technical grade spinosad. Check treatments were the Solbait formulation with no insecticide.

The field cages were certified by USDA-APHIS and Texas Department of Agriculture as secure for testing wild strains of fertile flies, but to simplify the mechanics of moving equipment and trees among cages, tests were performed with sterile strains of flies. Previous research (Mangan and Moreno, 1995) showed that sterile mass-reared Mexican fruit flies are equivalent to fertile flies in attraction and feeding behavior.

Tests were scheduled to run over 7 weekdays beginning on Monday. On the morning of the first day, four trees of each treatment (three age treatments and a check) were placed in the separate sections at the edge of the canopy of the mature tree. Immediately after placement of the trees, 3000 5–8 day old sterile Mexican fruit flies (both sexes) were released into each section. These flies had been maintained at room temperatures (22–23 °C, 50 ± 10% humidity) prior to release. Flies were released prior to 1000 h (CST). In some experiments (1 and 2) data were recorded on the first day, number of flies per tree and number of flies dead on the ground were counted at 1300 h. In all experiments on days 2–4 these counts were made during peak feeding periods (usually 0800 to 1000 h), mid-day, and mid-afternoon. Tests were originally designed to collect data from Monday afternoon after fly release until midday Friday. At noon on Friday, the nursery trees were removed from the cages and two plastic McPhail-type traps, baited with commercial Torula yeast pellets, were hung in the mature trees, one in the foliage proximal to the inner corner of the room and one near the outer corner. Traps were removed on Monday morning before beginning the next week of the experiment. In some experiments data collection periods were modified to omit samples of very low fly activity such as afternoon data during the summer months. Orchard maintenance activities such as irrigation also interfered with some tests and modifications were made.

2.1. Data collection and analysis

Counts of live flies on the trees, dead on the ground, and captured in traps were recorded and statistical analysis was performed using SYSTAT version 10.2.01. Recommendations for analysis were followed as described in Wilkinson et al. (1996) for examining main effects and interaction effects in multiway analysis of variance (ANOVA) models. The ANOVA results of each experiment is summarized including the F value, the degrees of freedom (d.f.) and the probability of significance for each treatment factor. For experiments having significant probabilities ($p<0.05$) for the complete ANOVA model, the Tukey HSD mean separation model was used to compare mean responses which are tabulated for each experiment.

3. Individual test methods and results

3.1. Experiment 1. Gf-120 bait longevity

The first test was designed to examine the persistance of the spray under Texas conditions. Sixteen potted trees were sprayed, 12 with GF 120 formulation at 80 mg l^{-1} AI spinosad and four trees with GF-120 bait without the insecticide as a check. At the end of 2 weeks we had trees with deposit ages of 1, 7 and 14 days as well as check trees (no insecticide) with deposits of these same ages. Sprayed trees were aged in an open green house with ambient air circulated by fans. Experiments were performed by selecting four trees from each of these age groups plus the four most recently sprayed check trees. Tests were performed in one of the field cage blocks of four individually caged mature trees. Four nursery trees from each age group were placed in each of these cage sections. Attraction of the bait, knockdown, and survival rate of the flies was recorded for each treatment. Tests were run from mid-September to mid-October 1999.

On Mondays, if rain was forecast for the next 5 days we did not begin tests but waited for clear forecasts. Spray ages therefore were not identical among tests, so for analysis treatment ages were grouped as less than 7 days (two tests), 10–20 days (three tests) and more than 20 days (three tests). Although the original design was for the experiment to be replicated three times with four treatments (check plus three age groups), weather interruptions forced us to follow a randomized design.

For analysis of these data, a large degree of heterogeneity in the variances of the data was noted. When the residuals from the ANOVA were plotted, we found that log transformations greatly reduced the heterogeneity. We plotted residuals against the estimated mean for each treatment, and after transformation the distributions became more symmetric and the magnitude of the residuals was more stable.

3.1.1. Results

Means, standard deviations and sample numbers for flies counted feeding on sprayed trees, dead flies collected, and flies trapped, in cages treated with the three bait ages and check are given in Table 1. Trapping numbers were recorded at the end of each trial, counts of dead flies and feeding flies were made three times per day. The three-factor ANOVA was used to compare numbers of flies attracted to trees and flies killed. Variation in counts of flies in trees differed significantly among the three times of collection per day ($F = 3.94$, d.f. $= 2, 138$, $p<0.034$) and day of the week ($F = 10.18$, d.f. $= 4, 138$, $p<0.001$). Counts of flies on trees differed significantly by time of spray, ages of spray, sprayed trees vs. the check ($F = 4.77$, d.f. $= 3, 138$, $p<.0.004$). When analyses were performed on the trap back rate

Table 1
Mean comparisons of numbers of flies in trees, numbers of flies counted for control (3-day old bait-no spinosad) and three age groups of sprayed trees (Experiment 1)

	Age group I <7 days	Age group II 10–20 days	Age group III 21–26 days	Check
Feeding flies				
Mean	45.68a	54.40ab	65.00b	70.83b
SD	55.62	53.38	51.95	54.51
N	25	50	36	37
Dead flies				
Mean	7.72a	8.97a	3.58ab	0.92b
SD	14.39	17.41	6.72	1.94
N	25	38	12	25
Trapped flies				
Mean	56.5*	121.75*	252.67	465.67
SD	2.12	106.55	246.79	124.23
N	2	4	2	3

Means (across rows) followed by followed by the same letter do not differ ($p>0.05$) by Tukey HSD mean comparison tests.
*Age groups I and II > check $p<0.06$.

(numbers of males and females trapped after 4.5 days exposure to sprayed trees) there was no significant difference among the three spray age groups. When the check was included with treatment in the analysis, the results showed that there were significant differences in fly recapture among the treatments ($F = 4.59$, d.f. = 3, 8, $p<0.038$).

Average numbers of live flies counted on trees was the highest in the check and the oldest treatment. Counts of dead flies in the cages, and numbers of surviving males and females trapped after a 4.5 day exposure indicated that mortality was reduced as the age of the spray on nursery trees increased. The numbers of surviving flies trapped after 4 days of exposure to treated trees was more than five times greater in the >21 day treatment than in the <7 day old treatment but this difference was not significant. The lack of significance in these differences was due to high variability in the older sprays. Flies trapped among the <7 day old treatments ranged from 21 to 27 females and 31 to 34 males. In the >21 day old treatment surviving flies trapped ranged from 37 to 231 males and 41 to 209 females.

3.2. Experiment 2. Laboratory cage tests, bait aging, spinosad concentration and toxicity

This was a test was to determine the effect of spray concentration on knockdown (mortality within 24 h of exposure) of flies at four spinosad concentrations. Standard GF 120 baits with no spinosad, and 8, 80, or 800 mg l^{-1} A.I. spinosad were applied to small trees at 3–4-day intervals and trees were placed in an open greenhouse. Leaves (trifoliates) were collected from each tree when spray groups had aged from 3 to 14 days. Age of spray was assigned into group I for 3 or 4 day, group II for 6–8 day and group III for 10–14 day old spray. Treated leaves were placed into cages with protein-starved, 8–12 day old fertile, flies supplied with 4% sugar water. Twenty-five males and 25 females were introduced into the cages and exposed to the treated leaves. Numbers of dead males and females were counted after 24 h exposure to the spray (Table 2).

3.2.1. Results

There were only three dead flies in the check sprays so these data are omitted from the analysis. The ANOVA results showed that bait concentration on the trees affected mortality of males ($F = 39.19$, d.f. = 2, 87, $p<0.001$) and females ($F = 31.18$, d.f. = 2,87, $p<.001$) when they were exposed to the sprayed leaves for 24 h. When the three spinosad concentrations are compared among aging treatments, the 80 and 800 ppm concentrations killed significantly more males and females than did the 8 ppm concentrations in the 3–5 day and the 6–10 day old baits. The 800 ppm killed significantly more males and females than the 8 and 80 ppm concentrations in tests with 11–14 day old baits. Comparisons of bait ages for each concentration showed no significant differences in female or male mortality for the three bait ages in the 8 or 800 ppm concentrations. In the 80 ppm concentration tests the 3–5 day old and 6–10 day old baits were significantly more effective in killing both sexes than the 11–14 day old baits.

3.3. Experiment 3. Insecticide dilution field cage test

For these tests four baits were tested consisting of the GF-120 formulation with 8, 80 or 800 mg l^{-1} concentrations of spinosad and check bait with no spinosad. Tests were carried out by spraying the nursery trees, and testing against flies for 2 weeks after bait droplets had congealed on the leaves. Testing was done from 15 October 1999 to 29 November 1999.

Formulation was applied to nursery trees using the methods previously described. Ambient humidity was variable during this time of year (October–November); for all four tests we waited for 3–4 h until bait droplets on leaves had dried to a firm, but still tacky spot, then we waited 2 weeks before beginning the test. On Monday before 0700 h, in each of the three cage sections we placed two trees with each of the three spinosad concentrations. The fourth cage section contained two check trees. Dead flies were collected and counts of live flies per tree were made at 1300 h on Monday and at 0900, 1100 and 1300 h each day until Friday. On Friday, after the 1300 h count the nursery trees were removed and two McPhail traps with hydrolyzed torula bait hung in the mature tree. Traps were removed at about

Table 2
Numbers of dead males and females

Age group		Dead males			Dead females		
		8 ppm	80 ppm	800 ppm	8 ppm	80 ppm	800 ppm
I	Mean	12.90a,1	20.20a,1 2	25.00a,2	13.60a,1	20.10a,1 2	24.80a,2
	SD	8.70	7.98	0	8.32	6.98	0.42
II	Mean	8.58a,1	22.83a,2	24.83a,2	10.17a,1	23.17a,2	24.67a,2
	SD	7.58	3.97	0.39	8.10	4.84	0.65
III	Mean	6.30a,1	9.80b,1	22.2a,2	7.70a,1	9.30b,1	21.40a,2
	SD	8.94	8.77	6.53	9.51	8.63	5.71

Spray droplets on leaves aged in an open green house then tested in laboratory cages for 24 h with 25 males or 25 females (Experiment 2).
Age groups I = bait age 3–5 days, II = bait age 6–10 days, III = bait age 11–14 days.
Age group (columns) followed by same letter not significantly different ($p>0.05$) by Tukey HSD test.
Concentrations (rows) followed by the same number not significantly different ($p>0.05$) by Tukey HSD test.

0700 the following Monday and trapped flies were counted.

3.3.1. Results

Fly counts from four sampling days, three sampling times, and three spinosad concentrations plus a control were tested for these three factors. The analysis of variance model included the effects of test (four replicates), toxicant concentration, day of treatment (4), and time of data collection (2). Interactions among these effects were also tested but only the interaction between day and concentration was significant for live flies counted in treated trees and dead flies collected. Toxicant concentration was a significant factor for numbers of flies feeding ($F = 5.19$, d.f. = 3,131, $p<.002$), total fly mortality ($F = 12.27$, d.f. = 3, 131, $p<0.001$), and total flies trapped ($F = 13.61$, d.f. = 3, 9, $p<0.001$). Mean separation statistics are given as letters following the means among the columns in Table 3. Comparisons of the means showed that the 8 mg l^{-1} concentration had significantly greater numbers of flies counted on the trees than the higher concentrations and was not significantly different from the check. Counts of dead males and females showed that the 800 mg l^{-1} was a superior treatment for killing both sexes. For flies surviving the treatments and being trapped, 80 and 800 mg l^{-1} concentrations were equivalent and significantly fewer males and females were trapped from 80 and 800 mg l^{-1} treatments than in the check or 8 mg l^{-1} concentration.

3.4. Experiment 4. Bait dilution test

In this test dilutions of the complete bait were compared. The GF-120 bait formulation is marketed as a concentrate that is diluted in the field with water so the spinosad concentration is 80 mg l^{-1} of bait. This is the recommended bait in this experiment. Two other dilutions were tested, 25% (1 part recommended GF-120: 3 parts water) concentration, and 10% (1 part recommended GF-120: 9 parts water) concentration (10 mg l^{-1} spinosad) and a check of the standard bait with no spinosad.

This experiment tested the effects of continuous aging of the bait dilutions over a 5-week period from 31 May to 6 July. Bait was applied to groups of 20 nursery trees as previously described, for each dilution. For each block of cages, two trees that received each dose were placed in each of the four cage sections. This provided two replicates in separate blocks of cages of each treatment per week. Trees were tested for 1 week, and then removed. In the following week another four treated trees were moved into each cage section. The test was run for 5 consecutive weeks. The spray on the trees at week 1 was 32 h old, trees placed in the field cage at week 5 had spray that was 704 h old. No trees were tested for more than a week in a cage.

Numbers of flies observed feeding on the nursery trees and numbers of dead flies on the cage floor were recorded three times per day (0800, 1100, and 1600 h) for 4 days in the four treatments over the 5 week period. Flies were trapped for 3 days with McPhail traps as in the previous field cage experiments. The feeding and mortality data consisted of 480 samples (three daily samples, four treatments, 4 days, 5 weeks, in two cages each with four sections).

3.4.1. Results

After the experiment began, samples at 1600 h provided very few flies on days with temperatures $>32\,^{\circ}C$ and foraging ants carried away nearly all moribund and dead flies in the afternoon. Our orchard management system scheduled irrigation on Fridays, so during weeks 2 and 4 the field cage was flooded and

Table 3
Means (standard deviations) and mean separation indicators[a] for counts of flies feeding on trees, dead flies collected and flies trapped after treatment for 3 concentrations of spinosad or check in GF-120 bait aged on trees in field cages (Experiment 3)

Treatment	Feeding flies	Total dead flies	Male survivors trapped	Female survivors trapped
0 (Check)	46.60 (52.40)b	3.05 (6.18)a	171.25 (70.32)a	175.25 (98.20)a
8 ppm	49.94 (54.56)b	0.955 (2.12)a	192.5 (97.50)a	243.25 (155.15)a
80 ppm	21.06 (23.99)a	3.96 (10.90)a	36.00 (27.31)b	40.50 (36.59)b
800 ppm	23.16 (29.55)a	45.66 (185.63)b	20.00 (25.02)b	23.75 (29.12)b

[a]Tukey HSD test, means followed by same letter do not differ ($p<0.05$).

dead flies could not be recovered. The final data set, therefore, had two daily samples (0800, 1100 h) from four treatments for 3 days (Tuesday–Thursday) in two blocks of cages over 5 weeks (240 samples).

The analysis of variance results were calculated for the four main effects, time of collection, day of collection, bait age, and bait concentration on number feeding and number of dead flies. These analyses indicated that the concentration of bait was a significant factor in counts of flies feeding on the treated trees ($F = 28.42$, d.f. $= 3$, 229, $P<0.001$) and numbers of dead flies collected in the cage ($F = 2.81$, d.f. $= 3$, 170, $P>.0.063$). The bait age was significant in counts of flies feeding ($F = 7.35$, d.f. $= 4$, 229, $P<.001$) and numbers of dead flies ($F = 3.69$, d.f. $= 4$, 170, $P<.001$). However, there was no pattern of increase or decrease in fly feeding rate or dead fly recovery over the duration of the experiment as shown in Table 4. Analysis of surviving flies trapped showed no significant differences among the bait concentrations (($F = 1.35$, d.f. $= 3$, 20, $P>0.063$) but significant differences among bait age ($F = 6.49$, d.f. $= 4$, 20, $P<.0.002$). As in the feeding and mortality data, there was no pattern of increase or decrease in fly survival over the 5 week duration of the experiment.

The summary of means and separations for these data (Table 4) show that there were significant differences among treatments for total numbers of flies feeding and total numbers of dead flies. The more dilute formulations had more feeding flies and fewer dead flies. Bait dilution did not significantly affect total numbers of surviving and trapped flies. Weekly counts of feeding and dead flies with progressively older baits did not have significant separation statistics and the trend of changes in numbers was not related to age of the bait. In these tests, treated nursery trees were aged in the greenhouse so previous weeks weather did not affect the bait condition. The second week of the test had the lowest mortality, largest numbers of flies feeding, and largest numbers of flies trapped for all the bait concentrations which may have been a result of irrigation of the orchard. But even when the data for this week are ignored there does not appear to be any clear change in bait function with age.

Table 4
Mean response of adult flies to dilutions of GF 120 bait for Baits aged 1 to 5 weeks in greenhouse before exposure to flies (Experiment 4)

Treatment	Week					
	1	2	3	4	5	Total (5 weeks)
Feeding flies						
Check	1162.5	553.5	950.0	431.0	626.5	3723.5a
8:1 dilution	729.0	198.0	793.5	732.0	426.5	2879ab
4:1 dilution	273.5	300.0	516.5	187.5	438.0	1715.5bc
Recommended bait	74.5	278.5	302.5	66.0	483.5	1205c
Adults dead						
Check	22.4	8.5	42.0	17.5	19.0	109.4a
8:1 dilution	32.5	10.5	56.0	40.0	22.5	161.5a
4:1 dilution	80.5	18.5	53.5	43.0	23.5	219ab
Recommended bait	243.5	13.5	189.5	111.0	26.5	584b
Adults recaptured						ns
Check	59.0	162.0	77.0	75.5	95.5	469
8:1 dilution	35.5	119.0	45.5	22.0	75.0	297
4:1 dilution	87.5	123.5	49.5	36.5	28.5	325.5
Recommended bait	10.5	158.0	29.0	30.5	36.5	264.5

ns: no significant difference among means.

4. Discussion

These tests examined the effects of dilution and environmental exposure of the GF-120 fruit fly bait on attraction and killing of Mexican fruit flies. The GF-120 bait was developed for use as a foliar spray. The bait attracts the flies, induces the flies to feed on the drops, and the spinosad kills the flies as a stomach poison. These experiments were carried out under field cage and laboratory conditions and mimicked the typical spray method in which bait is applied to spots on host plants or as bands of spray along rows of trees in orchards. Both cover sprays and spot sprays were designed to be applied as large (4–6 mm diameter) bait droplets which act as attractants. In these tests the bait lured flies from the canopy of mature trees onto nursery trees where they fed on the bait. The major aging effect was exposure to sunlight and drying of the bait in a greenhouse.

Daily patterns of flies feeding on check sprayed trees in the caged tree experiments indicated a gradual increase in numbers of flies feeding from days 1 through 4 then a slight decline on day 5. This slight decline may have been due to most of the control bait being consumed by the flies and ants. Numbers of flies counted on the trees with baits containing spinosad increased on days 1 and 2 but then declined on days 3, 4 and 5 as flies were killed by the spinosad.

Number of dead flies recorded allows comparison of relative toxicity of bait among treatments but underestimates the overall mortality. Dead fly count is actually an estimate of acute mortality because flies that lived more than 5 h after feeding would not be collected on the day they died. During the 2 or 4 h between dead fly collections, ants (*Solenopsis geminata* (Fabricius)) dissected and removed fly corpses. Ants could not be controlled without confounding pesticide effects. Large numbers of wings (>20) were found at the entrances to the ant nests during most of the morning counts. Although the numbers of dead flies collected were reduced by ants, we observed no evidence that ants biased the results among treatments. Treatments were rotated among cages and ant colonies were ubiquitous so that ants had access to all the cages.

The numbers of survivors trapped over the 2.5 day (weekend) period after 4.5 days of treatment provided a second method of evaluating bait efficacy. Trap captures were not affected by interference by ants. However, numbers of flies entering traps were dependent on fly foraging activity over a relatively short time period (normal trapping for pest management uses a 1 or 2 week sampling period). Both fly responses to the baits and to the traps were dependent on temperature, wind and humidity. In tests with treatments that were carried out in the same week, cages containing the greatest numbers of dead flies had the lowest numbers of flies trapped. However, when baits were compared in sequential weekly tests in experiment 4 (Table 4), the different ambient conditions from week to week masked possible effects of aging on the bait.

The series of trials in experiment 1 were carried out under less stressful weather conditions (September–October) than in experiment 4 (June–July). Statistical analysis (Table 2) showed only weak differences in performance relative to age of bait, but the increasing standard deviation suggests that the performance of the bait became less reliable as spray age increased.

The results of these experiments provide guidelines to determine bait dilution and spray interval for GF-120 bait. The results of the bait age experiments (1, 2, 4) indicate that with the recommended dilution of the bait (80 mg l^{-1} spinosad) the formulation reduces fly populations under field conditions for up to 20 days. The laboratory cage tests in experiment 3 show that older baits (>10 days field exposure) have reduced numbers of flies killed in 24 h. The dilution results indicate that changing the spinosad concentration in the bait (with the other ingredients unchanged) will reduce bait effectiveness when spinosad concentration is reduced from 80 to 8 mg l^{-1}. Increasing the spinosad concentration from 80 to 800 mg l^{-1} significantly increases knockdown (dead flies collected) but does not change fly attraction to the bait or numbers of survivors over a 4.5 day treatment period.

Dilution of the GF-120 bait by mixing the commercial formulation with water reduced attraction and knockdown, and allowed increased survival of flies. A fourfold reduction of bait concentration reduced average attraction of the flies, killing rate, and permitted greater average survival, but these differences were not significant. A 10-fold reduction of the bait significantly reduced these effectiveness factors in comparison with the undiluted bait mixture, and this bait was not significantly different from the nontoxic check. In theory the evaporation of water from the diluted baits should result in the same bait consistency as the recommended formulation. However, we observed that drying of more diluted baits resulted in reduced volume per drop and the drops appeared as flattened plaques on the leaf surface. Dilution apparently reduced the humectant properties of the bait resulting in a thin dry film on the leaf surface which was less attractive and more difficult for flies to ingest.

In the context of previously published tests of spinosad baits, these field cage experiments differ in methodology, factors tests and results. One major difference between these tests and those performed on *Ceratitis* and *Bactrocera* by the Hawaii group is in the time frames of testing. Revis et al. (2004) evaluated attractiveness and toxicity of two ages and three dilutions of GF120 based on 12 min exposure and observation. Barry et al. (2003) evaluated baits by placing flies on a leaf disc with a drop of bait then recording feeding time, or by recording fly mortality rate after feeding on the drops for a total time of 10 min. These time periods contrast with the experiments reported here which were carried out by recording activities and estimating survival over 3.5–4 day periods. Prokopy et al. (2004) tested cage emerged flies that were released at the edge of melon plots surrounded by four rows of sorghum receiving various treatments. Observations were made at half hour intervals for 9 h following release (8:30–16:30 h and 9:00–17:00 h). In field cage tests presented here, the flies showed little activity or migrated to the cage screens then responded to the sprayed plants the next morning.

Other reported trials used different bait formulations. For example field trial by Vargas et al. (2001) used a bait consisting of the formula published in Moreno and Mangan (1995). This formula in major components was nearly identical to GF120 but the protein source

(Mazoferm) is less purified and contains starch grains and other contaminants and residual sugars. The tests by Stark et al. (2004) used spinosad mixed in Provesta, a protein bait, previously tested in Hawaii. That test was carried out on cut coffee leaves placed on Petri plates in laboratory cages for 24 h. The tests in Vargas et al. (2003) used spinosad mixed with the attractants methyl eugenol or cuelure, which are used for male annihilation programs and are not comparable to protein baits.

The experiments reported here indicate that GF-120 fruit fly bait is most effective and persistent at the label concentrations. The bait may be diluted by 4:1 without significant loss of effectiveness; however, the knock-down effects are lost with dilution and after 10 days of aging in the field. The bait must be consumed by the insects for effective toxicity and because consumption of the bait by these fruit flies does not measurably occur under field conditions during the first few hours after exposure of the flies to the bait, measurable mortality may require several days. These observations suggest that this bait would be most effective when applied to immature adults so that such delays in feeding and mortality do not allow time for oviposition and product damage.

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